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The Content of Pantothenic Acid, Folic Acid and Vitamin B12 Like-Activity in Alfalfa Hay Grown in Utah Soils Treated with Different Fertilizers

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THE CONTENT OF PANTOTHENIC ACID, FOLIC ACID AND VITAMIN B_{12} LIKE-ACTIVITY IN ALFALFA HAY GROWN IN UTAH SOILS TREATED WITH DIFFERENT FERTILIZERS

by

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INTRODUCTION

Vitamins play an important role in the nutrition of man and animal. As early as 1897 Eijkman was able to deomonstrate the symptoms of thiamine deficiency disease in birds. Since then, vitamins have been regarded as one of the most essential factors in nutrition. Only small quantities in the diet are necessary, yet lack of any of them will inhibit the growth and produce certain kinds of deficiency diseases.

In planning a diet for man and animals, nutritionists must know with a degree of certainty the amount of vitamins present in certain kinds of foods and feed stuffs. It was essential that accurate methods for the determination of vitamins be developed. The microbiological methods were initiated by R. J. Williams (19) in 1919. Within the last few years, these methods have become widely used. At present, they occupy a very important position among the methods available for this purpose.

Importance

Since alfalfa has been recognized as the outstanding feedstuff, and about one-third of Utah's crop land and 38 percent of its irrigated land are planted to alfalfa, an increase in both the quantity and quality of alfalfa would increase the livestock production in this state.

This study is a part of a larger project conducted by the Utah State Agricultural Experiment Station dealing with the interrelationships between the composition of soils and plants and the nutrition of animals. The problem concerns the effect of fertilizers on the content of pantothenic acid, folic acid and vitamin B_{12} in alfalfa.

REVIEW OF LITERATURE

Little or no work has been done on the effect of fertilizers on the content of pantothenic acid, folic acid and vitamin B_{12} in alfalfa. There was, however, an evidence of direct correlation between the yield and the phosphorus content of the hay grown on soils treated with manure or super phosphate (11).

Pantothenic acid

Pantothenic acid was identified and named by R. J. Williams (15) in 1933. It is widely distributed in plant and animal tissues. The vitamin is required by microorganisms, rats, dogs, foxes, swine, fowls and other species of animals, has has been reported to be a specific growth factor for plants (5).

The chemical formula of pantothenic acid was proved to be Gamma di-hydroxy-beta, beta-dimethyl-butyro-beta-alanide (20).

Lipman et. al. (9) reported the presence of pantothenic acid in coenzyme A, which is required for the acetylation of aromatic amine in liver tissue. Riggs and Hegsted (14) found that rats rendered pantothenic acid-deficient acetylated only 50 percent of the amount excreted in 24 hours after a milligrams dose and 37 percent dose of p-aminobenzoic acid administered intraperitoneally as compared to the acetylation of 70 percent in normal rats.

Pantothenic acid deficiency symptoms vary in different species of animals. In chickens, the symptom is dermatosis. The vitamin is also important for hatchability and reproduction, but not for egg production (6).

Folic acids

The result of the deficiency of folic acid has first been observed as anemia in chicks by Hogan and Parrot (8). The Lederle Laboratory group (1) in 1945 successfully synthesized and identified the vitamins as the compounds formed by the attachment of various numbers of glutamic molecules to 4-[{(2-amino-4-hydroxy-6-pteridyl)-methyl}-amino]-benzoic acid, thus explaining the existence of different forms of folic acids.

Folic acids are recognized as required by the monkey, chick, turkey, fox, mink, dog and certain microorganisms.

Vitamin B12

The isolation of vitamin B_{12} in 1948 (12) (17) brought more world-wide interest and attention than other vitamins. The vitamin is effective in relieving the symptoms of pernicious anemia (21). Vitamin B_{12} is required by human beings and other monogastric animals for growth and hematopoiesis. Young calves also require vitamin B_{12} for growth.

Chemical and physical properties of vitamin B_{12} were established as having the empirical formula of $C_{61-64}H_{86-92}N_{14}O_{13}PCo$. The molecular weight is 1490 ± 150 (4).

Vitamin B_{12} is distributed in significant quantities mainly in animal proteins, and from this association the term Animal Protein was derived. Many types of microorganisms produce significant amounts of vitamin B_{12} and at the present time vitamin B_{12} is produced commercially by fermentation process.

The vitamin has a rather high melting point which is at least 300° C. It crystallizes as dark red needles which are very hygroscopic.

The vitamin appeares to be a cobalt coordination complex having six groups about the carbon atom (13). Degradation of vitamin B_{12} with acid gives 5, 6-dimethylbenzimidazole (6).

Microbiological assay for vitamin B_{12} like-activity in alfalfa using L. Leichmanii as the test organism yielded the values from 12 to 45 milligrams ($\times 10^{-5}$) per 100 grams of dehydrated alfalfa meal (3).

METHOD OF PROCEDURE

Plan of work

Areas were established on farms in Cache, Duchesne, Emery, San Juan, Uintah and Utah counties of Utah. Each area was divided into plots of 18'x 75' except in Duchesne County where they were 15'x 90'.

Each farm had seven different fertilizer treatments and a control with no fertilizer. There were four replications of treated and untreated plots arranged at random.

The fertilizers were added to the soil in the spring of 1947 or 1948 and Ranger alfalfa was then seeded. Three hundred and fifty-two samples were collected during the season of 1949.

The determination of pantothenic acid, folic acid and vitamin B_{12} in the hay samples collected from the plots mentioned above was carried out in the chemistry laboratory of Utah State Agricultural College Experiment Station.

The purpose of this study is to determine the content of pantothenic acid, folic acid and vitamin B_{12} like-activity in alfalfa hay grown in plots treated with different fertilizers in different counties of Utah.

Methods of analysis

Pantothenic acid was determined by microbiological assay of Skeggs and Wright (16) using Lactobacillus arabinosus 17-5 as the test organism and Mylase P was made, using this enzyme for liberating free pantothenic acid from the bound form.

Since later work of Neiland and Strong (10) showed that chicken liver enzyme fortified with intestinal phosphatase gave a maximum

liberation of the free vitamin, assay of several samples at random has been made by using these enzymes and the results are compared to those liberated by Mylase P.

Folic acid and vitamin B_{12} were determined by microbiological assay using Lactobacillus caseii and Lactobacillus leichmannii ATCC No. 4797 respectively as the test microorganisms.

Since other factors which are present in natural products such as desoxynucleotides can replace vitamin B_{12} in promoting growth of certain Lactobacillus microorganisms (15), and no reliable microbiological assay method for analyzing the true activity of vitamin B_{12} is available, the assay has been made to determine the vitamin B_{12} like-activity in alfalfa hay.

The growth of the organisms was measured turbidimetrically at 650 millimicrons on a Junior Coleman spectophotometer as suggested by the Association of Vitamin Chemists (2). The contents of the vitamin were estimated by comparing the optical density read with a standard curve using standard U.S.P. calcium pantothenate, folic acid and crystalline vitamin B_{12} respectively.

The methods used for analysis of the data are described by Snedecor (18). The data were analyzed only with respect to variation in treatment and replication. No attempt was made to analyze the data with respect to various locations.

The least significant difference between treatment means were calculated. The coefficients of variations were also calculated.

Table 1. Reproducibility of Analytical Values for Pantothenic Acid in Alfalfa Hay by Microbiological Assay Method

Sample No.	Series 1	Series 2	Average of replications	Deviation from the mean	Percent Deviation
225 B	4.07	3.83	3.95	0.07	1.77
275 B	3.89	4.00	3.94	0.06	1.52
325 B	3.36	3.36	3.36	0.00	0.00
375 B	3.86	3.75	3.80	0.05	1.32
425 B	3.96	4.07	3.96	0.01	0.25
475 B	4.14	3.99	4.06	0.07	1.72
525 B	4.03	3.88	4.00	0.02	0.50
575 B	3.72	3.64	3.68	0.04	1.09
582 B	4.58	4.45	4.51	0.06	1.33
603 B	4.02	3.96	3.99	0.03	0.76

Average percentage deviation from the mean 1.63

Analysis of Variance

Source of Variation	d.f. (1)	Mean Square
Between samples	9	0.1722
Between duplicates within samples	10	0.0090
Total	19	

(1) Degree of freedom

Table 2. Reproducibility of Analytical Values for Folic Acid in Alfalfa Hay by Microbiological Assay Method

Values in milligrams per hundred grams					
Sample No.	Series 1	Series 2	Average of replications	Deviation from the mean	Percent deviation
267 B	0.512	0.501	0.506	0.006	1.19
300 B	0.678	0.683	0.681	0.003	0.43
321 B	0.650	0.650	0.650	0.000	0.00
370 B	0.620	0.600	0.610	0.010	1.64
398 B	0.563	0.550	0.557	0.006	1.08
427 B	0.575	0.525	0.550	0.025	4.55
475 B	0.600	0.670	0.635	0.035	5.51
497 B	0.563	0.550	0.557	0.007	1.26
527 B	0.632	0.630	0.631	0.001	0.16
586 B	0.688	0.682	0.685	0.003	0.44

Average percentage deviation from the mean 1.63

Analysis of Variance

Source of Variation	d.f. (1)	Mean Square
Between samples	9	0.0073
Between duplicates within samples	10	0.0050
Total	19	

(1) Degree of freedom

Table 3. Reproducibility of Analytical Values for Vitamin B_{12} Like-activity in Alfalfa Hay by Microbiological Assay Method

	Values in milligrams x 10^{-5} per hundred grams				
Sample No.	Series 1	Series 2	Average of replications	Deviation from the mean	Percent deviation
206 B	0.182	0.173	0.178	0.004	2.25
263 B	0.129	0.133	0.131	0.002	1.53
312 B	0.182	0.190	0.186	0.004	2.15
334 B	0.129	0.112	0.121	0.008	6.61
427 B	0.124	0.130	0.127	0.003	2.36
479 B	0.258	0.243	0.251	0.007	2.79
508 B	0.230	0.218	0.224	0.006	2.75
535 B	0.142	0.134	0.138	0.004	2.90
586 B	0.160	0.165	0.163	0.002	1.23
600 B	0.138	0.148	0.143	0.005	3.49

Average percentage deviation from the mean 2.81

Analysis of Variance

Source of Variation	d.f. (1)	Mean Square
Between samples	9	0.0382
Between duplicates within samples	10	0.0005
Total	19	

(1) Degree of freedom

Table 4. Comparison of Values of Pantothenic Acid Content Obtained by Using Mylase P and by Chicken Liver Fortified with Intestinal Phosphatase

Values in milligrams per hundred grams				
Sample No.	Value by Mylase P	Value by chicken liver and intestinal phosphatase	Difference	Percent Difference
225 B	3.73	5.87	2.14	36.46
375 B	3.75	5.86	2.11	36.01
525 B	3.89	6.43	2.66	41.37
602 B	3.70	5.96	2.26	37.92
604 B	4.14	5.95	1.81	30.42

Average percent difference 36.44

RESULTS AND DISCUSSION

Presentation of the Analytical Data

Pantothenic acid

Pantothenic acid values using Mylase P as the enzyme are presented in tables 5-10 inclusive, on the basis of the different counties. The sources of variation are discussed below.

Table 4 shows the difference in values of pantothenic acid content obtained from using Mylase P and by chicken liver enzymes fortified with intestinal phosphatase. The average values of pantothenic acid in alfalfa hay obtained by using chicken liver enzyme fortified with intestinal phosphatase were about 36 percent higher than the values obtained by using Mylase P. This is due to the more complete liberation of the vitamin by the chicken liver enzyme than the Mylase P.

Replication. There is a slight difference between replications, but it is of no statistical significance.

Treatment. In most plots there was no significant difference in pantothenic acid content of fertilized and unfertilized plots except in the second and third crops of alfalfa grown in plots on Tuttle farm in Emery County.

Counties. There appeared to be a slight difference in the amount of pantothenic acid with respect to different counties and also a difference between the different locations in the same county. The lowest average concentration of pantothenic acid, 3.29 milligrams per 100 grams, was found in the second crop in Utah County. The highest average concentration, 4.47 milligrams per 100 grams, was found in the third crop of Cache County. The concentrations in other counties are intermediate

between the lowest and the highest values.

Folic acid

The analytical data of folic acid are presented in tables 11-16 inclusive, on the basis of the different counties. The sources of variation are discussed below.

Replication. There is a slight difference between replications, but it is of no statistical significance.

Treatment. There was a difference in treatments in most plots. Highly significant differences were obtained in the first and third crops of plots in Cache County but not in the second crop. This is probably due to the variation in sampling and period of harvesting. Highly significant differences were also obtained in both the first and second crops in Uintah County. There were significant differences in the plots in the third crop in Emery County; also in the second and third crops of Utah County and the second crop in Duchesne County.

Counties. There were differences in the amount of folic acid with respect to different counties and also a variation between the difference in location in the same county. The lowest average concentration of folic acid, 0.472 milligrams per 100 grams, was found in the second crop of Utah County. The highest concentration, 0.838 milligrams per 100 grams, was found in the first crop of San Juan County. The concentrations of folic acid in other counties were intermediate between the highest and the lowest.

Vitamin B_{12}

The analytical data of vitamin B_{12} are presented in tables 17-21 inclusive, on the basis of the different counties. The sources of variation are discussed below.

Replication. No statistical significant differences between replications were obtained.

Treatment. Highly significant differences were obtained in every crop of alfalfa hay in every county in Utah.

Counties. There were differences in the amount of vitamin B_{12} with respect to different counties and also a variation between the different locations in the same county. The lowest average concentration of vitamin B_{12} like-activity, 0.120×10^{-5} milligrams per hundred grams dry basis, was found in the first crop on Prott Lyman farm, Monticello, San Juan County. The highest average concentration, 0.259×10^{-5} milligrams per hundred grams, was found in the second crop on Tuttle farm, Castle Dale, Emery County.

Table 5. Pantothenic Acid Content of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots on Wilson Farm, Petersboro, Cache County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8-10 tons cattle manure	8-10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in milligrams per hundred grams on dry basis							
Replication	Second Crop							
1	3.36	4.15	3.86	3.86	4.32	3.98	3.43	3.94
2	4.03	3.89	4.16	3.91	4.21	3.94	3.85	4.36
3	4.38	2.91	3.84	4.13	4.16	3.91	3.96	3.85
4	4.18	4.49	4.41	4.08	3.94	3.82	4.12	4.22
Average	3.99	3.86	4.07	3.99	4.16	3.91	3.84	4.09
Replication	Third Crop							
1	3.85	4.42	4.43	3.83	4.24	3.83	4.21	4.25
2	4.13	4.51	4.68	4.31	4.03	3.91	4.63	4.15
3	4.69	3.91	4.46	4.13	4.21	4.15	3.55	3.76
4	4.61	3.80	4.31	4.63	3.89	3.86	4.14	4.25
Average	4.32	4.16	4.47	4.23	4.09	3.94	4.13	4.10

(1) tsp = treble super phosphate; P_2O_5, 42%; CaO, 21.8% and some trace elements

(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre

Table 6. Pantothenic Acid Content of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots on Abbot Farm, Duchesne County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8-10 tons cattle manure	8-10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in milligrams per hundred grams on dry basis							
Replication	Second Crop							
1	4.45	4.12	4.15	4.04	3.83	4.18	3.64	4.08
2	4.38	3.30	3.97	3.87	4.54	3.89	3.87	4.09
3	3.86	4.34	3.89	3.89	4.04	3.86	4.10	3.66
4	3.81	3.96	3.85	3.82	3.89	4.28	3.76	4.14
Average	4.10	3.93	3.97	3.91	4.08	4.05	3.84	3.99

(1) tsp = treble super phosphate; P_2O_5, 42%; CaO, 21.8% and some trace elements
(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre

Table 7. Pantothenic Acid Content of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots in San Juan County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8-10 tons cattle manure	8-10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in milligrams per hundred grams on dry basis							
	Carhart farm, Dove Creek							
Replication	First Crop							
1	3.95	3.85	3.48	4.07	3.95	3.92	3.81	3.97
2	3.70	4.10	3.52	4.31	3.84	3.77	3.48	3.99
3	3.84	4.09	3.78	3.88	3.95	3.73	3.98	3.84
4	3.76	3.97	3.51	3.74	3.59	4.29	4.35	4.10
Average	3.81	4.00	3.57	4.00	3.83	3.68	3.91	3.98
	Prott Lyman farm, Monticello							
Replication	First Crop							
1	3.91	3.44	3.78	3.89	3.42	3.20	4.01	3.74
2	4.06	3.68	3.72	4.24	3.92	3.74	3.41	4.14
3	3.69	3.89	4.01	3.02	3.49	3.44	3.19	3.44
4	4.21	3.76	3.42	3.99	3.20	3.78	3.70	3.78
Average	3.97	3.69	3.73	3.79	3.51	3.54	3.58	3.78

(1) tsp = treble super phosphate; P_2O_5, 42%; CaO, 21.8% and some trace elements
(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre

Table 8. Pantothenic Acid Content of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots in Uintah County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8-10 tons cattle manure	8-10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in milligrams per hundred grams on dry basis							
	Roy Taylor farm, Randlett							
Replication	First Crop							
1	4.52	3.84	4.01	4.51	4.02	3.95	4.20	4.20
2	4.10	4.12	4.42	4.27	4.12	4.15	4.16	3.88
3	4.39	3.84	4.10	4.20	3.82	4.11	3.79	4.14
4	4.27	3.72	3.90	3.93	4.16	4.14	4.33	4.02
Average	4.32	3.88	4.11	4.23	4.03	4.09	4.12	4.06
	Orson Nielson farm, Vernal							
Replication	Second Crop							
1	4.30	3.95	3.36	3.41	4.15	4.18	4.19	4.02
2	3.76	4.41	3.56	4.34	3.33	3.90	3.43	4.34
3	4.32	4.21	3.52	4.34	3.26	4.16	4.38	3.39
4	3.81	3.98	3.90	4.12	3.90	3.43	3.26	4.37
Average	4.05	4.14	3.59	4.05	3.66	3.92	3.82	4.03

(1) tsp = treble super phosphate; P_2O_5, 42%; CaO, 21.8% and some trace elements

(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre

Table 9. Pantothenic Acid Content of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots on R. J. Evans Farm, Lehi, Utah County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8–10 tons cattle manure	8–10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in Milligrams per hundred grams on dry basis							
Replication	Second Crop							
1	3.73	2.84	3.60	3.96	3.96	3.67	4.32	3.22
2	3.72	3.11	3.70	4.16	3.77	3.85	3.58	3.35
3	4.05	3.36	3.81	3.00	2.87	3.80	3.66	4.02
4	3.80	3.85	3.69	3.83	2.85	3.43	4.13	3.90
Average	3.83	3.29	3.70	3.74	3.36	3.69	3.92	3.62
Replication	Third Crop							
1	4.17	3.88	3.81	3.98	3.86	3.75	4.42	3.99
2	3.50	4.37	4.00	4.23	3.90	4.24	4.35	3.74
3	4.05	3.41	3.72	4.06	3.11	3.83	4.19	4.27
4	3.84	4.44	4.13	4.29	3.97	3.91	4.64	4.23
Average	3.89	4.03	3.92	4.14	3.71	3.93	4.40	4.06

(1) tsp = treble super phosphate; P_2O_5, 42%; CaO, 21.8% and some trace elements.
(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre

Table 10. Pantothenic Acid Content of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots on Tuttle Farm, Castle Dale, Emery County, 1949

	a	b	c	d	e	f	g	h
	129 lbs tsp (1)	258 lbs tsp	516 lbs tsp	516 lbs tsp plus 100 lbs $CuSO_4$	516 lbs tsp plus 12-15 tons chicken manure	12-15 tons chicken manure	516 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1947)							
	Value in milligrams per hundred grams on dry basis							
Replication	Second Crop							
1	4.26	4.50	3.41	3.53	4.11	3.86	3.79	4.43
2	4.22	4.34	3.57	3.81	4.40	3.84	4.28	4.45
3	4.48	3.95	3.62	3.80	4.26	3.85	3.42	4.30
4	4.49	4.24	3.40	4.24	3.69	3.81	3.43	4.43
Average	4.36	4.26	3.50	3.85	4.10	3.84	3.73	4.40
Replication	Third Crop							
1	4.47	3.42	4.39	4.23	3.44	4.21	3.80	3.74
2	4.17	3.82	4.18	4.49	3.51	3.81	4.20	4.04
3	4.14	4.25	4.33	4.65	3.62	3.95	4.30	4.16
4	3.86	3.94	3.81	3.90	2.92	3.82	3.72	4.29
Average	4.16	3.86	4.18	4.32	3.37	3.95	4.01	4.06

(1) tsp = treble super phosphate, available P_2O_5, 42%; CaO, 21.8% and some trace elements.
(2) Borax, 48.4 lbs; cobaltous chloride, 24.2 lbs; copper sulfate, 48.4 lbs; and manganese sulfate, 98.6 lbs per acre

Table 11. Folic Acid Content of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots on Wilson Farm, Petersboro, Cache County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8-10 tons cattle manure	8-10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in milligrams per hundred grams on dry basis							
Replication	Second Crop							
1	0.637	0.402	0.641	0.513	0.632	0.603	0.550	0.623
2	0.628	0.623	0.623	0.527	0.575*	0.654	0.512	0.612
3	0.578	0.597	0.617	0.517	0.572	0.647	0.703	0.583
4	0.603	0.602	0.613	0.602	0.513	0.595	0.642	0.604
Average	0.612	0.556	0.624	0.540	0.573	0.625	0.602	0.606
Replication	Third Crop							
1	0.542	0.514	0.421	0.513	0.602	0.512	0.612	0.714
2	0.531	0.638	0.632	0.528	0.631	0.474	0.713	0.602
3	0.632	0.679	0.627	0.621	0.579	0.302	0.728	0.608
4	0.621	0.595	0.612	0.671	0.632	0.531	0.703	0.702
Average	0.582	0.607	0.573	0.583	0.611	0.455	0.689	0.657

(1) tsp = treble super phosphate; P_2O_5, 42%; CaO, 21.8% and some trace elements

(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre

* Estimated value

Table 12. Folic Acid content of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots on Abbot Farm, Duchesne County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8-10 tons cattle manure	8-10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in milligrams per hundred grams on dry basis							
Replication	Second Crop							
1	0.632	0.631	0.721	0.921	0.521	0.578	0.502	0.550
2	0.712	0.612	0.547	0.782	0.631	0.712	0.563	0.620
3	0.702	0.549	0.678	0.635	0.721	0.632	0.612	0.575
4	0.698	0.693	0.542	0.721	0.678	0.610	0.604	0.531
Average	0.686	0.621	0.622	0.765	0.638	0.633	0.570	0.569

(1) tsp = treble super phosphate; P_2O_5, 42%; CaO, 21.8% and some trace elements
(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre

Table 13. Folic Acid Content of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots in San Juan County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8-10 tons cattle manure	8-10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in milligrams per hundred grams on dry basis							
	Carhart farm, Dove Creek							
Replication	First Crop							
1	0.628	0.621	0.620	0.517	0.521	0.721	0.623	0.838
2	0.514	0.611	0.620	0.623	0.723	0.600	0.517	0.980
3	0.523	0.597	0.518	0.503	0.623	0.421	0.725	0.723
4	0.613	0.513	0.513	0.600	0.624	0.590	0.613	0.810
Average	0.570	0.586	0.568	0.561	0.623	0.583	0.620	0.838
	Prott Lyman farm, Monticello							
Replication	First Crop							
1	0.713	0.601	0.513	0.724	0.418	0.550	0.721	0.623
2	0.621	0.702	0.677	0.513	0.513	0.612	0.614	0.713
3	0.613	0.628	0.598	0.618	0.578	0.634	0.513	0.512
4	0.607	0.519	0.617	0.621	0.525	0.618	0.518	0.412
Average	0.639	0.613	0.601	0.619	0.509	0.604	0.592	0.565

(1) tsp = treble super phosphate; P_2O_5, 42%; CaO, 21.8% and some Trace elements

(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre

Table 14. Folic Acid Content of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots in Uintah County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8-10 tons cattle manure	8-10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in milligrams per hundred grams on dry basis							
	Roy Taylor farm, Randlett							
Replication	First Crop							
1	0.525	0.478	0.518	0.717	0.602	0.779	0.625	0.412
2	0.539	0.465	0.523	0.706	0.623	0.767	0.568	0.501
3	0.520	0.515	0.514	0.587	0.614	0.632	0.613	0.428
4	0.608	0.477	0.520	0.672	0.635	0.746	0.602	0.412
Average	0.548	0.484	0.519	0.671	0.619	0.731	0.602	0.438
	Orson Nielson farm, Vernal							
Replication	Second Crop							
1	0.620	0.528	0.620	0.427	0.518	0.650	0.831	0.407
2	0.617	0.523	0.623*	0.572	0.524	0.678	0.765	0.398
3	0.597	0.535	0.628	0.581	0.547	0.623	0.742	0.423
4	0.613	0.517	0.613	0.568	0.533	0.732	0.739	0.447
Average	0.612	0.526	0.621	0.537	0.531	0.671	0.769	0.419

(1) tsp = treble super phosphate; P_2O_5, 42%; CaO, 21.8% and some trace elements
(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre
* Estimated value

Table 15. Folic Acid Content of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots on R. J. Evans Farm, Lehi, Utah County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8-10 tons cattle manure	8-10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in milligrams per hundred grams on dry basis							
Replication	Second Crop							
1	0.550	0.621	0.713	0.618	0.423	0.719	0.523	0.721
2	0.525	0.613	0.618	0.579	0.514	0.603	0.500	0.621
3	0.623	0.614	0.600	0.575	0.528	0.430	0.740	0.630
4	0.524	0.628	0.612	0.517	0.423	0.421	0.519	0.621
Average	0.556	0.619	0.636	0.572	0.472	0.543	0.571	0.648
Replication	Third Crop							
1	0.543	0.623	0.543	0.600	0.550	0.587	0.551	0.551
2	0.407	0.547	0.621	0.612	0.512	0.563	0.521	0.421
3	0.521	0.523	0.678	0.702	0.587	0.542	0.623	0.550
4	0.603	0.514	0.625	0.628	0.509	0.423	0.518	0.525
Average	0.519	0.552	0.617	0.636	0.540	0.529	0.553	0.512

(1) tsp = treble super phosphate; P_2O_5, 42%; CaO, 21.8% and some trace elements.
(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre.

Table 16. Folic Acid Content of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots on Tuttle Farm, Castle Dale, Emery County, 1949

	a	b	c	d	e	f	g	h
	129 lbs tsp (1)	258 lbs tsp	516 lbs tsp	516 lbs tsp plus 100 lbs $CuSO_4$	516 lbs tsp plus 12-15 tons chicken manure	12-15 tons chicken manure	516 lbs tsp plus trace elements (2)	Control, no Fertilizer added
	(Treatments per acre - applied in spring, 1947)							
	Value in milligrams per hundred grams on dry basis							
Replication	Second Crop							
1	0.421	1.070	0.542	0.412	0.523	0.621	0.512	0.512
2	0.532	0.621	0.514	0.428	0.507	0.536	0.531	0.478
3	0.548	0.607	0.527	0.517	0.603	0.613	0.517	0.520
4	0.563	0.568	0.613	0.600	0.545	0.607	0.563	0.511
Average	0.516	0.717	0.549	0.489	0.545	0.594	0.531	0.505
Replication	Third Crop							
1	0.625	0.573	0.655	0.452	0.623	0.611	0.512	0.732
2	0.514	0.621	0.623	0.527	0.618	0.628	0.500	0.611
3	0.507	0.523	0.675	0.513	0.517	0.619	0.550	0.514
4	0.513	0.550	0.510	0.510	0.579	0.677	0.632	0.622
Average	0.540	0.567	0.616	0.501	0.584	0.634	0.549	0.620

(1) tsp = treble super phosphate, available P_2O_5, 42%; CaO, 21.8% and some trace elements

(2) Borax, 48.4 lbs; cobaltous chloride, 24.2 lbs; copper sulfate, 48.4 lbs; and manganese sulfate, 98.6 lbs per acre

Table 17. Vitamin B_{12} Like-activity of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots in Uintah County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8-10 tons cattle manure	8-10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in milligrams per hundred grams on dry basis ($\times 10^{-5}$)							
	Roy Taylor farm, Randlett							
Replication	First Crop							
1	.227	.202	.112	.142	.203	.212	.112	.123
2	.225	.198	.163	.148	.214	.232	.127	.137
3	.242	.195	.142	.150	.227	.207	.129	.135
4	.229	.189	.163	.152	.242	.226	.132	.129
Average	.231	.196	.145	.148	.222	.219	.125	.131
	Orson Nielson farm, Vernal							
Replication	Second Crop							
1	.187	.202	.230	.143	.213	.123	.152	.132
2	.163	.208	.227	.158	.210	.128	.148	.142
3	.178	.199	.242	.168	.220	.130	.149	.138
4	.182	.210	.232	.120	.217	.126	.139	.142
Average	.178	.205	.233	.147	.215	.127	.147	.139

(1) tsp = treble super phosphate; P_2O_5, 42%; CaO, 21.8% and some trace elements.
(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre.

Table 18. Vitamin B_{12} Like-activity of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots on R. J. Evans Farm, Lehi, Utah County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8-10 tons cattle manure	8-10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in milligrams per hundred grams on dry basis ($\times 10^{-5}$)							
Replication	Second Crop							
1	.179	.127	.202	.120	.173	.202	.240	.202
2	.187	.135	.210	.125	.168	.189	.245	.214
3	.183	.157	.207	.124	.167	.193	.238	.207
4	.186	.142	.198	.127	.169	.195	.242	.263
Average	.184	.140	.204	.124	.167	.195	.241	.222
Replication	Third Crop							
1	.127	.135	.138	.120	.147	.128	.230	.137
2	.135	.137	.140	.123	.152	.132	.245	.145
3	.129	.128	.127	.125	.152	.127	.262	.142
4	.130	.129	.135	.124	.150	.125	.252	.137
Average	.130	.132	.135	.123	.150	.123	.247	.140

(1) tsp = treble super phosphate; P_2O_5, 42%; CaO, 21.8% and some trace elements.

(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre.

Table 19. Vitamin B_{12} Like-activity of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots on Abbot Farm, Duchesne County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8-10 tons cattle manure	8-10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in milligrams per hundred grams on dry basis ($\times 10^{-5}$)							
Replication	Second Crop							
1	.220	.152	.178	.220	.240	.167	.187	.169
2	.242	.148	.167	.210	.250	.154	.175	.138
3	.237	.147	.123	.209	.262	.152	.162	.157
4	.217	.149	.142	.218	.242	.159	.158	.124
Average	.229	.149	.153	.214	.250	.158	.171	.147

(1) tsp = treble super phosphate, P_2O_5, 42%; CaO, 21.8% and some trace elements.
(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre

Table 20. Vitamin B_{12} Like-activity of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots in San Juan County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8-10 tons cattle manure	8-10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in milligrams per hundred grams on dry basis ($\times 10^{-5}$)							
	Carhart farm, Dove Creek							
Replication	First Crop							
1	.178	.123	.152	.121	.128	.230	.127	.210
2	.182	.142	.143	.112	.132	.224	.132	.219
3	.185	.132	.127	.123	.144	.217	.127	.208
4	.180	.142	.143	.143	.127	.224	.123	.200
Average	.181	.135	.141	.124	.133	.224	.127	.209
	Prott Lyman farm, Monticello							
Replication	First Crop							
1	.230	.108	.128	.182	.228	.212	.145	.127
2	.227	.123	.112	.178	.227	.203	.135	.132
3	.214	.127	.123	.168	.218	.227	.132	.125
4	.242	.120	.140	.172	.225	.208	.143	.132
Average	.228	.120	.126	.175	.225	.213	.139	.124

(1) tsp = treble super phosphate, P_2O_5, 42%; CaO, 21.8% and some trace elements

(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre

Table 21. Vitamin B_{12} Like-activity of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots on Tuttle Farm, Castle Dale, Emery County, 1949

	a	b	c	d	e	f	g	h
	129 lbs tsp (1)	258 lbs tsp	516 lbs tsp	516 lbs tsp plus 100 lbs $CuSO_4$	516 lbs tsp plus 12-15 tons chicken manure	12-15 tons chicken manure	516 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied spring, 1947)							
	Value in milligrams per hundred grams on dry basis ($\times 10^{-5}$)							
Replication	Second Crop							
1	.173	.181	.227	.137	.135	.187	.263	.127
2	.167	.178	.243	.142	.137	.172	.254	.130
3	.169	.178	.237	.147	.139	.178	.264	.132
4	.178	.180	.240	.142	.142	.174	.258	.135
Average	.147	.179	.237	.142	.131	.178	.259	.131
Replication	Third Crop							
1	.154	.121	.201	.153	.142	.142	.232	.214
2	.163	.127	.198	.150	.145	.147	.227	.205
3	.167	.125	.195	.148	.143	.138	.243	.221
4	.164	.125	.203	.142	.138	.144	.241	.232
Average	.162	.125	.199	.148	.142	.143	.237	.218

(1) tsp = treble super phosphate, available P_2O_5, 42%; CaO, 21.8% and some trace elements

(2) Borax, 48.4 lbs; cobaltous chloride, 24.2 lbs; copper sulfate, 48.4 lbs; and manganese sulfate, 98.6 lbs per acre

Table 22. Vitamin B_{12} Like-activity of Ranger Alfalfa Hay Produced on Fertilized and Unfertilized Plots on Wilson Farm, Petersboro, Cache County, 1949

	a	b	c	d	e	f	g	h
	200 lbs tsp (1)	400 lbs tsp	400 lbs tsp plus $CuSO_4$	100 lbs $CuSO_4$	400 lbs tsp plus 8-10 tons cattle manure	8-10 tons cattle manure	400 lbs tsp plus trace elements (2)	Control, no fertilizer added
	(Treatments per acre - applied in spring, 1948)							
	Value in milligrams per hundred grams on dry basis (x 10^{-5})							
Replication	Second Crop							
1	.127	.132	.212	.142	.240	.182	.139	.123
2	.132	.125	.212	.128	.229*	.176	.142	.132
3	.124	.138	.227	.147	.220	.169	.136	.126
4	.129	.137	.260	.158	.242	.173	.134	.124
Average	.128	.133	.228	.144	.176	.175	.133	.126
Replication	Third Crop							
1	.125	.237	.187	.120	.150	.210	.187	.223
2	.129	.243	.172	.127	.162	.205	.175	.187
3	.122	.258	.168	.132	.146	.214	.173	.229
4	.130	.253	.142	.125	.154	.230	.179	.232
Average	.131	.248	.167	.126	.153	.215	.179	.215

(1) tsp = treble super phosphate, P_2O_5, 42%; CaO, 21.8% and some trace elements
(2) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs; and zinc sulfate, 35 lbs per acre
* Estimated value

Table 23. Analysis of Variance of Pantothenic Acid Content of Ranger Al Counties of Utah, 1949

Sources of Variation	d.f. (1)	Cache Petersboro Second Crop	Cache Petersboro Third Crop	Duchesne Duchesne Second Crop	MEAN San Duchesne Dove Creek First Crop
Treatment	7	0.0514	0.1033	0.0357	0.0815
Replication	3	0.1512	0.0546	0.0236	0.0077
Error	21	0.1155	0.0954	0.0790	0.0502
Total	31				
L.S.D. (2)		0.090	0.454	0.042	
Treatment/acre (3)		Treatment Means (Values in milli			
200 lbs tsp (4)		3.99	4.32	4.10	3.81
400 lbs tsp		3.86	4.16	3.93	4.00
400 lbs tsp plus 100 lbs $CuSO_4$		4.07	4.47	3.97	3.57
100 lbs $CuSO_4$		3.99	4.23	3.91	4.00
400 tsp plus 8-10 tons cattle manure		4.16	4.09	4.08	3.83
8-10 tons cattle manure		3.91	3.94	4.05	3.68
400 tsp plus trace elements (5)		3.84	4.13	3.87	3.91
Control, no fertilizer added		4.09	4.10	3.99	3.98
Average of plots		3.99	4.18	3.99	3.85

(1) Degree of freedom
(2) Least significant mean difference at 0.05 probability level
(3) Fertilizers applied in spring, 1948
(4) Treble super phosphate, P_2O_5 42%; CaO, 21.8% and some trace elements
(5) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs
* Statistically significant at 0.05 probability level
** Statistically highly significant at 0.01 probability level

falfa Hay Produced on Fertilized and Unfertilized Plots in Different

SQUARES (s^2)				
Juan	Uintah		Utah	
Monticello	Randlett	Vernal	Lehi	
First Crop	First Crop	Second Crop	Second Crop	Third Crop
0.0932	0.0686	0.1614	0.1881	0.1662
0.1608	0.0272	0.195	0.0198	0.1692
0.0913	0.0351	0.1783	0.1600	0.0722
0.445	0.276	0.606	0.588	0.395
grams per hundred grams dry basis)				
3.97	4.32	4.05	3.83	3.89
3.69	3.88	4.14	3.29	4.03
3.73	4.11	3.59	3.70	3.92
3.79	4.23	4.05	3.74	4.14
3.51	4.03	3.66	3.36	3.71
3.54	4.09	3.92	3.69	3.93
3.58	4.12	3.82	3.92	4.40
3.78	4.06	4.03	3.62	4.06
3.70	4.11	3.94	3.52	4.01

zinc sulfate, 35 lbs per acre

Table 24. Analysis of Variance of Folic Acid Content of Ranger Alfalfa Of Utah, 1949

Sources of Variation	d.f. (1)	Cache Petersboro Second Crop	Cache Petersboro Third Crop	Duchesne Duchesne Second Crop	MEAN San Dove Creek First Crop
			**	*	**
Treatment	7	0.00400	0.01914	0.01614	0.03357
Replication	3	0.00100	0.00867	0.00033	0.00767
Error	21	0.00333	0.00471	0.00605	0.00657
Total	31				
L.S.D. (2)		0.077	0.101	0.114	0.119
Treatment/acre (3)					Treatment Means (Values in milli
200 lbs tsp (4)		0.612	0.582	0.686	0.570
400 lbs tsp		0.556	0.607	0.621	0.586
400 lbs tsp plus 100 lbs $CuSO_4$		0.624	0.573	0.622	0.568
100 lbs $CuSO_4$		0.540	0.583	0.765	0.561
400 tsp plus 8-10 tons cattle manure		0.573	0.640	0.638	0.623
8-10 tons cattle manure		0.625	0.455	0.633	0.583
400 lbs tsp plus trace elements (5)		0.602	0.689	0.570	0.620
Control, no fertilizer added		0.606	0.657	0.569	0.838
Average of plots		0.592	0.595	0.638	0.619

(1) Degree of freedom
(2) Least significant mean difference at 0.05 probability level
(3) Fertilizers applied in spring, 1948
(4) Treble super phosphate, P_2O_5, 42%; CaO, 21.8% and some trace elements
(5) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs
* Statistically significant at 0.05 probability level
** Statistically highly significant at 0.01 probability level

Hay Produced on Fertilized and Unfertilized Plots in Different Counties

SQUARES (s^2)				
San Juan	Uintah		Utah	
Monticello	Randlett	Vernal	Lehi	
First Crop	First Crop	Second Crop	Second Crop	Third Crop
	**	**	*	*
0.00643	0.03814	0.04557	0.01314	0.00814
0.00633	0.00167	0.00067	0.00900	0.00633
0.00662	0.00162	0.00138	0.00528	0.00300
	0.059	0.055	0.107	0.080
grams per hundred grams dry basis)				
0.639	0.548	0.612	0.556	0.519
0.613	0.484	0.526	0.619	0.552
0.601	0.519	0.621	0.636	0.617
0.619	0.671	0.537	0.572	0.636
0.509	0.619	0.531	0.472	0.540
0.604	0.731	0.671	0.543	0.529
0.592	0.602	0.769	0.571	0.553
0.565	0.438	0.419	0.648	0.512
0.593	0.577	0.586	0.577	0.577

zinc sulfate, 35 lbs per acre

Table 25. Analysis of Variance of Vitamin B_{12} Like-activity of Ranger Counties of Utah, 1949

Sources of Variation	d.f. (1)	Cache Petersboro Second Crop	Cache Petersboro Third Crop	Duchesne Duchesne Second Crop	MEAN San Dove Creek First Crop
		**	**	**	**
Treatment	7	0.00782	0.00795	0.00660	0.00628
Replication	3	0.0016	0.00006	0.00035	0.00002
Error	21	0.00010	0.00015	0.00016	0.00007
Total	31				
L.S.D. (2)		0.015	0.018	0.019	0.013
Treatment/acre (3)					Treatment Means (Values in milli
200 lbs tsp (4)		0.128	0.130	0.229	0.181
400 lbs tsp		0.133	0.248	0.149	0.135
400 tsp plus 100 lbs $CuSO_4$		0.228	0.167	0.153	0.141
100 lbs $CuSO_4$		0.144	0.126	0.214	0.124
400 tsp plus 8-10 tons cattle manure		0.176	0.153	0.250	0.133
8-10 tons cattle manure		0.175	0.215	0.158	0.224
400 lbs tsp plus trace elements (5)		0.133	0.179	0.171	0.127
Control, no fertilizer added		0.126	0.215	0.147	0.139
Average of plots		0.157	0.179	0.184	0.151

(1) Degree of freedom
(2) Least significant mean difference at 0.05 probability level
(3) Fertilizers applied in spring, 1948
(4) Treble super phosphate, P_2O_5, 42%; CaO, 21.8% and some trace elements
(5) Borax, 50 lbs; cobaltous chloride, 35 lbs; manganese sulfate, 100 lbs
* Statistically significant at 0.05 probability level
** Statistically highly significant at 0.01 probability level

Alfalfa Hay Produced on Fertilized and Unfertilized Plots in Different

SQUARES (s^2)				
Juan	Uintah		Utah	
Monticello	Randlett	Vernal	Lehi	
First Crop	First Crop	Second Crop	Second Crop	Third Crop
**	**	**	**	**
0.00877	0.00783	0.00632	0.00625	0.00667
0.00006	0.000410	0.00007	0.00013	0.00005
0.00007	0.00016	0.0009	0.00013	0.00003
0.012	0.016	0.014	0.017	0.008
grams per hundred grams dry basis) ($\times 10^{-5}$)				
0.228	0.231	0.178	0.184	0.130
0.120	0.198	0.205	0.140	0.132
0.126	0.145	0.233	0.204	0.135
0.175	0.148	0.147	0.124	0.123
0.225	0.222	0.215	0.167	0.150
0.213	0.219	0.127	0.195	0.123
0.209	0.125	0.147	0.241	0.247
0.124	0.131	0.139	0.222	0.140
0.178	0.177	0.174	0.185	0.148

zinc sulfate, 35 lbs per acre

Table 26. Analysis of Variance of Pantothenic Acid, Folic Acid and Vitamin B_{12} Like-activity Content of Ranger Alfalfa on Fertilized and Unfertilized Plots at Castle Dale, Emery County, Utah, 1949

		Sources of Variation				
		Treatment	Replication	Error	Total	L.S.D. (2)
		7	3	21	31	
Mean squares (s^2) 2nd crop	Pantothenic	0.4265**	0.0486	0.0547		0.344
	Folic	0.0210	0.00567	0.0098		
	Vitamin B_{12}	0.00871**	0.00017	0.00002		0.007
Mean squares (s^2) 3rd crop	Pantothenic	0.3295**	0.2118	0.0590		0.357
	Folic	0.00843*	0.0030	0.00333		0.085
	Vitamin B_{12}	0.00663**	0.00003	0.00004		0.009

Values in milligrams per hundred grams on dry basis

	Treatment per acre (3)	129 lbs tsp (4)	250 lbs tsp	516 lbs tsp	516 lbs tsp plus 100 lbs $CuSO_4$	516 lbs tsp plus 12-15 tons chicken manure	12-15 tons chicken manure	516 lbs tsp plus trace elements (5)	Control, no fertilizer added	Aver. of plots
Mean squares (s^2) 2nd crop	Pantothenic	4.36	4.26	3.50	3.85	4.10	3.84	3.73	4.40	4.01
	Folic	0.516	0.717	0.549	0.489	0.545	0.594	0.531	0.505	0.556
	Vitamin B_{12} (x 10^{-5})	0.147	0.179	0.237	0.142	0.131	0.178	0.259	0.131	0.176
Mean squares (s^2) 3rd crop	Pantothenic	4.16	3.86	4.18	4.32	3.37	3.95	4.01	4.06	3.99
	Folic	0.540	0.567	0.616	0.501	0.584	0.634	0.549	0.620	0.576
	Vitamin B_{12} (x 10^{-5})	0.162	0.125	0.199	0.148	0.142	0.143	0.237	0.218	0.172

(1) Degree of freedom
(2) Least significant mean difference at 0.05 probability level
(3) Fertilizers applied in spring, 1947
(4) Treble super phosphate, P_2O_5, 42%; CaO, 21.8% and some trace elements
(5) Borax, 48.4 lbs; $CoCl_2$, 24.2 lbs; $CuSO_4$, 48.4 lbs; $MnSO_4$, 98.6 lbs per acre
* Statistically significant at 0.05 probability level
** Statistically highly significant at 0.01 probability level

SUMMARY AND CONCLUSION

1. Three hundred and fifty-two different samples of alfalfa hay grown on fertilized and unfertilized plots in Cache, Duchesne, Emery, San Juan, Uintah and Utah counties of Utah were collected during the weason of 1949.

2. Determination of samples mentioned above for the content of Pantothenic acid, folic acid, and vitamin B_{12} like-activity were made by microbiological methods.

3. In general, there were statistically significant differences in the content of folic acid and vitamin B_{12} like-activity in Ranger alfalfa hay of the same crop in the same location due to the fertilizer treatment. There appeared to be no significant difference in the content of Pantothenic acid of the same crop except in the second and third crops of alfalfa in plots in Emery County where there were highly significant differences.

4. The effect of any one fertilizer treatment was not consistent from crop to crop, county to county, or location to location in the same county. No conclusion as to the effect of any definite type of fertilizer on the content of pantothenic acid, folic acid, and vitamin B_{12} like-activity can be made.

5. The lowest average concentration of pantothenic acid, 3.39 milligrams per 100 grams, was found in the second crop in Utah County. The highest average concentration, 4.47 milligrams per 100 grams, was found in the third crop in Cache County.

6. The lowest average concentration of folic acid, 0.472 milligrams per 100 grams, was found in the second crop in Utah County. The highest concentration, 0.838 milligrams per 100 grams, was found in the first crop in San Juan County.

7. The lowest average concentration of vitamin B_{12} like-activity, 0.12×10^{-5} milligrams per hundred grams, was found in the first crop in San Juan County. The highest average concentration, 0.259×10^{-5} milligrams per 100 grams, was found in the second crop of Emery County.

8. The variation in folic acid and vitamin B_{12} like-activity in alfalfa hay was undoubtedly due to the different fertilizer treatment.

9. The over-all statistical non significant difference in the content of pantothenic acid in alfalfa with respect to treatment is probably due to some other factors such as the variation in sampling and some error in analysis.

10. Significant differences in folic acid values were obtained in eight out of eleven alfalfa crops analyzed. The addition of fertilizers appears to be significant in increasing the folic acid value in seven crops of alfalfa out of eleven analyzed.

11. Significant differences in vitamin B_{12} like-activity were obtained in all alfalfa crops analyzed. The addition of fertilizer appears to increase the vitamin B_{12} like-activity in the nine crops out of eleven analyzed.

12. Further investigation on this subject will be required to determine other causes of the variation in the content of these vitamins.

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